GENETIC STRUCTURE AND DIFFERENTIATION WITHIN THE EASTERN SPOTTED SKUNK (*SPILOGALE PUTORIUS*): A MICROSATELLITE ANALYSIS

A Thesis

Presented to the

Faculty of the College of Graduate Studies and Research of

Angelo State University

In Partial Fulfillment of the

Requirements for the Degree

MASTER OF SCIENCE

by

ALEXANDRA ANNMARIE SHAFFER

August 2017

Major: Biology

GENETIC STRUCTURE AND DIFFERENTIATION WITHIN THE EASTERN SPOTTED SKUNK (*SPILOGALE PUTORIUS*): A MICROSATELLITE ANALYSIS

by

ALEXANDRA ANNMARIE SHAFFER

APPROVED:

Dr. Robert C. Dowler

Dr. Loren K. Ammerman

Dr. Nicholas J. Negovetich

Dr. Twyla J. Tasker

Dr. Susan E. Keith

27 June 2017

APPROVED:

Dr. Susan E. Keith
Dean of the College of Graduate Studies and Research

ACKNOWLEDGMENTS

The amount of people that contributed to the success of this project is almost innumerable. Without the instruction, support, knowledge, generosity, and encouragement from my advisors, Dr. Dowler and Dr. Ammerman, this project would not have been possible. I cannot thank them enough for their guidance and assistance both in the field and in the lab. Their endless support truly enabled the project to culminate as it did. In addition, the support and guidance from my committee members, Dr. Negovetich, Dr. Tasker, and Dr. Keith, is greatly appreciated. For all that each of you did to contribute to this project, whether it was improving my statistical analysis or motivating and encouraging me through my Master's program, I thank you. Many more are to thank within the Angelo State Biology Department for their support, especially Jody Casares, who was instrumental in helping me order necessary reagents and supplies (usually last minute on rush order), who forever reminded me to submit my timesheet (so as to get paid for all the time spent working on this project), and who showed genuine support for myself and my graduate project. Additionally, although much of my project was lab-based, a large component involved extensive field work, which would not have been possible without my field partner, Clint Perkins. Together we endured 100 days in the field which included navigating through fields and groves of "secret cactus" and greenbriar, narrowly missing stepping on several venomous snakes, accumulating hundreds, but more likely thousands, of mosquito, chigger, fire ant, and tick bites, tent camping in the midst of severe wind and thunderstorms, demolishing 6 car tires, and last, but not least, numerous car breakdowns on the highway and on muddy roads, culminating in the seizing of Clint's truck's engine. Despite all the headaches we endured, I

think our California-Louisiana alliance worked well in the end, as this project would have been entirely different without him.

None of this research would have been possible without the financial assistance afforded by the Texas Comptroller's Office, which funded travel costs, supplies, and my research assistantship. Also, I would like to acknowledge the 4 members of our technical advisory panel who donated their time and resources throughout the project to help ensure its completion and adherence to the contract with the Comptroller's office: Jerry Dragoo, John Karges, Damon Lesmeister, and Amie Treuer-Kuehn. Additionally, grants awarded to me by the Head of the River Ranch and the Graduate Research Fellowship through Angelo State supported the broadening of my thesis and enabled me to share my research at numerous conferences throughout the course of my graduate program.

Of course, my genetic analysis would not have been possible without the loaned and donated tissues acquired from various organizations and individuals, including: Abilene Christian University Natural History Collection; Angelo State Natural History Collections; Anniston Museum of Natural History; Campbell Museum of Natural History; Dickinson College Natural History Teaching Collection; Texas A&M Biodiversity Research and Teaching Collections; Museum of Texas Tech University Genetic Resources Collection; Museum of Wildlife and Fish Biology; James Krupa and University of Kentucky Vertebrate Teaching Collection; Tina Hannon and the Florida Fish and Wildlife Conservation Commission; Andrew Edelman, Ty Sprayberry, Cody Cornelison and University of West Georgia; Matthew Gompper and University of Missouri; Nikole Castleberry and Georgia Museum of Natural History; Geriann Albers and North Carolina Wildlife Resources

Commission; Blake Sasse and Arkansas Game and Fish Commission; Jerry Herbst; Molly McDonough and Adam Ferguson; and Jason Strickland.

Additionally, I am grateful to the many landowners and organizations that allowed access to their property to conduct field surveys: Rob Denkhaus and staff at the Fort Worth Nature Center; Jon Demboski, Barrett Durst, and staff at Lake Somerville State Park; Sid Richardson Scout Ranch; Matthew Schnupp and King Ranch, Wesley Newman and the Katy Prairie Conservancy, Charles Pekins and Fort Hood, and Daniel Walker and Powderhorn Ranch. Unfortunately, due to contract restraints I am unable to disclose the names of the private landowners, but the hospitality that was provided by all really did make all the difference during my 10 days in the field. Fred Collins also deserves a sincere "thank you" and recognition, as his recommendations for several survey locations led us to a majority of our spotted skunk detections. This field work would also not have been possible without the assistance from many volunteers who donated entire weekends or more of their time for the joy of catching (or attempting to catch) spotted skunks: James Amani, Megan Dory, Zac Ellsworth, Tristan Fielder, Bonnie Gulas-Wroblewski, Malorri Hughes, Kaitlynn LeBrasseur, Autumn Long, Gerardo Lorta, Tim Maddox, Marissa McEligot, Pete Ortega, Raymond Robbins, Kendra Saaranen, Dr. Ben Skipper, Dustin Tarrant, Devika Tijerina, and Craig Tipton.

Last but not least, I have my family to thank for their love, support, and encouragement throughout this process. A special thank you to my fiancé, Garrett Gibson, for moving almost halfway across the country to support me in every way possible while I worked on achieving my Master's. Thank you for putting up with my continued absence from San Angelo during the field season and for your unfailing support even after my

malodorous encounters with skunks. This thesis is as much mine as it is yours. Finally, thank you to my mom, dad, and brothers who supported me from afar during this process. This accomplishment would not have been possible without all of you.

ABSTRACT

The fluctuating nature of eastern spotted skunk (*Spilogale putorius*) populations over the past century has prompted concern over their conservation status, especially since this species is encountered infrequently and is relatively understudied. Although *S. putorius* is regarded as vulnerable by the International Union for Conservation of Nature, with the plains subspecies, *S. p. interrupta*, being considered for endangered species status, the genetic diversity and structure of the species is unknown. To enable genetic comparisons among the 3 subspecies, as well as to test the validity of the subspecies designations, tissue samples (n = 81) were analyzed across 11 cross-species microsatellite loci. Structure analyses indicated the presence of 3 clusters commensurate with morphological subspecies designations. The minimal gene flow and strong genetic differentiation ($F_{ST} > 0.195$) present among subspecies indicate the need to consider each as a unique evolutionarily significant unit, as these genetic differences could reflect behavioral, physiological, or habitat differences.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

INTRODUCTION

The eastern spotted skunk, *Spilogale putorius*, is an uncommon mesocarnivore native to the central and eastern United States with a geographic range extending from Tamaulipas, Mexico to southern Pennsylvania, and an east-west distribution from the Continental Divide to southern Florida (Kinlaw 1995). Three subspecies of the eastern spotted skunk are currently recognized: the plains spotted skunk (*S. p. interrupta*), which is distributed largely throughout the Great Plains of the central and midwestern United States, the Appalachian spotted skunk (*S. p. putorius*), which occurs throughout the eastern United States and is generally associated with the Appalachian Mountain range, and the Florida spotted skunk (*S. p. ambarvalis*), which is restricted to peninsular Florida (Kinlaw 1995). Morphologically, all 3 subspecies retain the same striping pattern, yet differences in the width of these stripes, and therefore the relative ratio of black to white, serve to differentiate them (Van Gelder 1959). Specifically, *S. p. interrupta* exhibits the least amount of white overall, as noted by the thinner, white dorsal and shoulder stripes, a smaller, triangular nose patch, and the reduction or absence of white hairs present in the distal tip of the tail (Van Gelder 1959). In contrast, *S. p. ambarvalis* displays the greatest amount of white overall, as noted by the presence of thicker stripes, a larger nose patch, and the greater presence of white at the tip of the tail (Van Gelder 1959). In addition, adult males of the Florida subspecies attain the smallest average weight (400 g), in comparison to adult males of the Appalachian (600 g) and plains (660 g) subspecies (Van Gelder 1959).

Although this mephitid was formerly considered a common carnivore in the midwestern United States, the eastern spotted skunk, and more specifically the plains

subspecies, has experienced pronounced population declines throughout its range since the 1940s (Choate et al. 1973; Kaplan and Mead 1991; Gompper and Hackett 2005). Despite its past prevalence in the fur trade (annual multi-state harvests >100,000), overharvesting has not been implicated in the decline of eastern spotted skunk populations (Gompper and Hackett 2005). Instead, the large-scale changes in agricultural practices that occurred throughout the 20th century are the most likely contributors to the observed population declines. Specifically, the modernization of farming methods is hypothesized to have been the primary impetus for the observed declines, as the destruction of dilapidated farm buildings, fence rows, creek bottoms, and wood piles (habitats historically abundant with spotted skunks) for industrial farming purposes served to reduce habitat and prey availability (Crabb 1948; Kaplan and Mead 1991; Gompper and Hackett 2005). Although it is agreed that anthropogenic activity instigated and hastened the decline of this skunk in the 1940s, human-related activity is also thought to have facilitated the range expansion and local population size increases of the eastern spotted skunk during the late 19th century. For example, the then-marshy plains of the central United States were drained for farming efficiency, thereby enabling colonization of a previously uninhabitable area, and farm house and outbuilding construction provided shelter for the skunks while affording them a steady food source in the form of crops, crop-eating insects, and commensal rodents (Van Gelder 1959; Choate et al. 1973).

The fluctuating nature of eastern spotted skunk populations over the past century has prompted concern over their conservation status, especially since this species is encountered very infrequently and is relatively understudied. Therefore, it is widely acknowledged that the eastern spotted skunk requires further population monitoring across its entire range.

Currently, there is a paucity of studies aimed at assessing the status of local populations of eastern spotted skunks (Choate et al. 1973; Boppel and Long 1994; Reed and Kennedy 2000), with only a few focused on detecting (Hackett et al. 2007; Hardy 2013) or determining habitat requirements (McCullough and Fritzell 1984; Reed and Kennedy 2000; Lesmeister et al. 2008, 2009, 2013) for this elusive mephitid. In response to the documented population declines and lack of *S. putorius* sightings, the International Union for Conservation of Nature (IUCN) now regards the eastern spotted skunk as vulnerable (Gompper and Jachowski 2016). Additionally, the U.S. Fish and Wildlife Service is currently considering the plains spotted skunk for listing as federally endangered (USFWS Federal Register 2012). Furthermore, on a state-by-state basis, the eastern spotted skunk is considered endangered, threatened, imperiled, or as a species of greatest conservation need in many states throughout its range (Eastern Spotted Skunk Cooperative Study Group 2017).

Despite the plethora of federal and state-level conservation status designations, there remains an absence of genetic data for the entire species. Genetic markers, such as microsatellites, are especially useful when researching rare and understudied species, as they are capable of amplifying homologous sequences in closely related taxa, thus eliminating the need to develop *de novo* markers on a species-by-species basis. A multitude of studies have validated the use of these nuclear markers across species boundaries and have been successful in addressing topics relating to the genetic variability and differentiation of populations, conservation, and hybridization (Kyle et al. 2004; Grobler et al. 2005; Floyd et al. 2011; McManus et al. 2015). Specifically within *Spilogale*, cross-species microsatellites have been utilized by Floyd et al. (2011) to determine genetic differentiation within and among mainland western spotted skunks (*S. gracilis*) and island spotted skunks (*S. g.*

amphiala) and by Jones et al. (2013) to determine the spatial and genetic organization of the island spotted skunk. However, microsatellites, nor any other molecular marker, have ever been used on eastern spotted skunks, according to extensive literature searches.

Therefore, the objectives of this study were threefold: (1) determine the genetic variability of the plains spotted skunk using microsatellite markers, (2) compare the genetic variability of the plains spotted skunk to that of the Appalachian and Florida spotted skunks, and (3) test the validity of the 3 eastern spotted skunk subspecies designations using molecular techniques, as morphological differences among them are the only metric currently supporting their distinction.

MATERIALS AND METHODS

Sample collection (plains spotted skunk). —From October 2015–May 2016 and October 2016–January 2017, we conducted field surveys for the plains spotted skunk throughout the state of Texas. Ten counties were surveyed (Burleson, Calhoun, Coryell, Harris, Kleberg, Navarro, Tarrant, Waller, Wichita, and Wise counties), with sampling lasting 7 days at each location. We anesthetized (with a 10 mg/kg dose of ketamine) live-trapped individuals (Tomahawk Live Trap, Hazelhurst, WI) in order to: (1) ascertain the overall condition, sex, and reproductive status, (2) obtain standard museum measurements, (3) collect ectoparasites, urine, and fecal samples when possible, (4) affix a unique, identifying ear tag (National Band and Tag Co., Newport, KY), (5) and acquire a 2 mm ear clip from the distal tip of the pinna for genetic analysis (Talbot et al. 2012; Jones et al. 2013). We stored ear clips in liquid nitrogen until they could be transferred to a -80˚C freezer for permanent storage. All trapped individuals were handled following the American Society of Mammalogists' guidelines for the use of wild animals in research (Sikes et al. 2016), and all sampling protocols were approved by the Angelo State University Institutional Animal Care and Use Committee (IACUC Approval No: 15-15).

Sample collection (all subspecies). —To supplement the number of individuals obtained by field surveys, and to obtain tissue from the Appalachian and Florida spotted skunks, tissue samples representing all eastern spotted skunk subspecies were requested from museum collections, when available (Table 1, Fig. 1). Other sources of genetic material included the salvaging of road-killed animals and obtaining individuals from fur trapper harvests. In addition, samples from non-vouchered specimens were obtained via donations from researchers throughout the United States (Table 2). A majority of these donations were

Table 1.—Vouchered specimens of *Spilogale putorius* examined for this study including the respective tissue and catalog numbers at each housing facility, the tissue type from which genomic DNA was extracted, the morphological subspecies identification, sex, and general collection information. Museum collection acronyms are as follows: ACUNHC (Abilene Christian University Natural History Collection), AMNH (Anniston Museum of Natural History), ASNHC or ASK (Angelo State Natural History Collections), CMNH (Campbell Museum of Natural History), DCNHTC (Dickinson College Natural History Teaching Collection), GMNH (Georgia Museum of Natural History), MWFB (Museum of Wildlife and Fish Biology), MWSU (Midwestern State University), NCSM (North Carolina Museum of Science), TCWC (Texas A&M Biodiversity Research and Teaching Collections), TTU or TK (Museum of Texas Tech University Genetic Resources Collection), UKVTC (University of Kentucky Vertebrate Teaching Collection), UMBMC (University of Missouri Bird and Mammal Collection), and UNSM (University of Nebraska State Museum). Tissue abbreviations: E (Ear), H (Heart), HK (Heart or Kidney), K (Kidney), L (Liver), Mu (Muscle), T (Toe pad). NA = Not available, U = Unknown.

Tissue no.	Catalog no.	Organization	Tissue	Subspecies	Sex	State	County	Collection date
ACC1139	ACC1139	CMNH	Mu	*putorius*	M	SC	Oconee	25-Dec-2006
ACUNHC1957	ACUNHC1957	ACUNHC	L	*interrupta*	M	TX	Taylor	10-Feb-2016
ASK4529	ASNHC10229	ASNHC	L	*interrupta*	F	TX	Bell	6-Aug-1996
ASK4856	ASNHC11774	ASNHC	L	*interrupta*	F	TX	Coryell	20-May-1996
ASK4858	ASNHC11773	ASNHC	K	*interrupta*	F	TX	Coryell	24-Nov-1996
ASK6142	ASNHC13370	ASNHC	L	*interrupta*	M	TX	Coleman	21-Mar-2003
ASK6824	ASNHC13369	ASNHC	HK	*interrupta*	M	TX	Brown	22-Feb-2004
ASK7225	ASNHC13371	ASNHC	L	*interrupta*	M	TX	Milam	17-Apr-2005
ASK7809	ASNHC13372	ASNHC	L	*interrupta*	M	TX	Taylor	23-Apr-2007
ASK7814	TCWC59601	TCWC	HK	*interrupta*	M	TX	Waller	18-Mar-2005
ASK7874	ASNHC13555	ASNHC	HK	*interrupta*	M	TX	Harris	16-Apr-2008
ASK7931	ASNHC13554	ASNHC	L	*interrupta*	M	TX	Waller	16-Dec-2008
ASK9618	ASNHC14653	ASNHC	Mu	*interrupta*	U	TX	Jack	29-Mar-2011
ASK9654	ASNHC14878	ASNHC	Mu	*interrupta*	U	TX	Robertson	15-Mar-2011
ASK9686	ASNHC14891	ASNHC	L	*interrupta*	M	TX	Harris	19-Mar-2004
ASK11870	NA	ASNHC	L	*interrupta*	M	SD	Brule	27-Mar-2017

Table 1.—Continued

Tissue no.	Catalog no.	Organization	Tissue	Subspecies	Sex	State	County	Collection date
ASK11871	NA	ASNHC	L	*interrupta*	M	SD	Brule	2-Apr-2017
ASK11872	NA	ASNHC	L	*interrupta*	M	SD	Brule	27-Mar-2017
ASK11873	NA	ASNHC	L	*interrupta*	M	TX	Wichita	16-Apr-2017
ASK11881	NA	UNSM	L	*interrupta*	NA	NE	Cherry	20-Feb-2017
ASK11884	NA	ASNHC	K	*interrupta*	NA	TX	Waller	3-Apr-17
ASK11911	DCNHTC329	DCNHTC	L	*putorius*	U	GA	Marion	31-Mar-2015
ASK11914	NA	UMBMC	Mu	*interrupta*	NA	AR	U	U
ASK11915	NA	UMBMC	Mu	*interrupta*	NA	AR	Scott	19-Feb-2016
ASK11916	NA	ACUNHC	L	*interrupta*	M	TX	Taylor	9-Feb-2017
ASK12461	NA	ASNHC	L	*interrupta*	M	SD	Brule	Fall 2015
ASK12462	NA	ASNHC	L	*interrupta*	M	SD	Brule	Fall 2015
ASK12466	NA	AMNH	L	*putorius*	M	AL	Cleburne	7-Feb-2015
ASK12467	NA	GMNH	H	*putorius*	M	GA	Towns	11-Apr-2015
ASK12468	NC 2016-001	NCSM	H	*putorius*	M	NC	Graham	25-Feb-2016
ASK12491	NA	ASNHC	E	*interrupta*	U	TX	Brazos	21-Oct-2015
JJK3648	JJK3648	UKVTC	T	*putorius*	M	KY	Clay	31-Mar-2016
JJK3857	JJK3857	UKVTC	L	*putorius*	U	KY	McCrerry	4-Mar-2017
TCWC60748	TCWC60748	TCWC	NA	*interrupta*	M	TX	Harris	14-Apr-2009
TK29908	MWSU22686	TTU	L	*interrupta*	F	TX	Archer	18-Jan-1991
WFB8979	WFB8979	MWFB	Mu	*putorius*	U	AL	Lee	U

Fig. 1.—Map indicating the collection locality of eastern spotted skunks (n = 81) utilized in microsatellite analyses. From small to large, circles represent sample sizes of n = 1, n = 2–3, n = 4–5, n = 9–11, and n = 24. Subspecies ranges are outlined in black and are color coded (cross-hatched) according to the respective subspecies (blue = *S. p. interrupta*, orange = *S. p. putorius*, gray = *S. p. ambarvalis*).

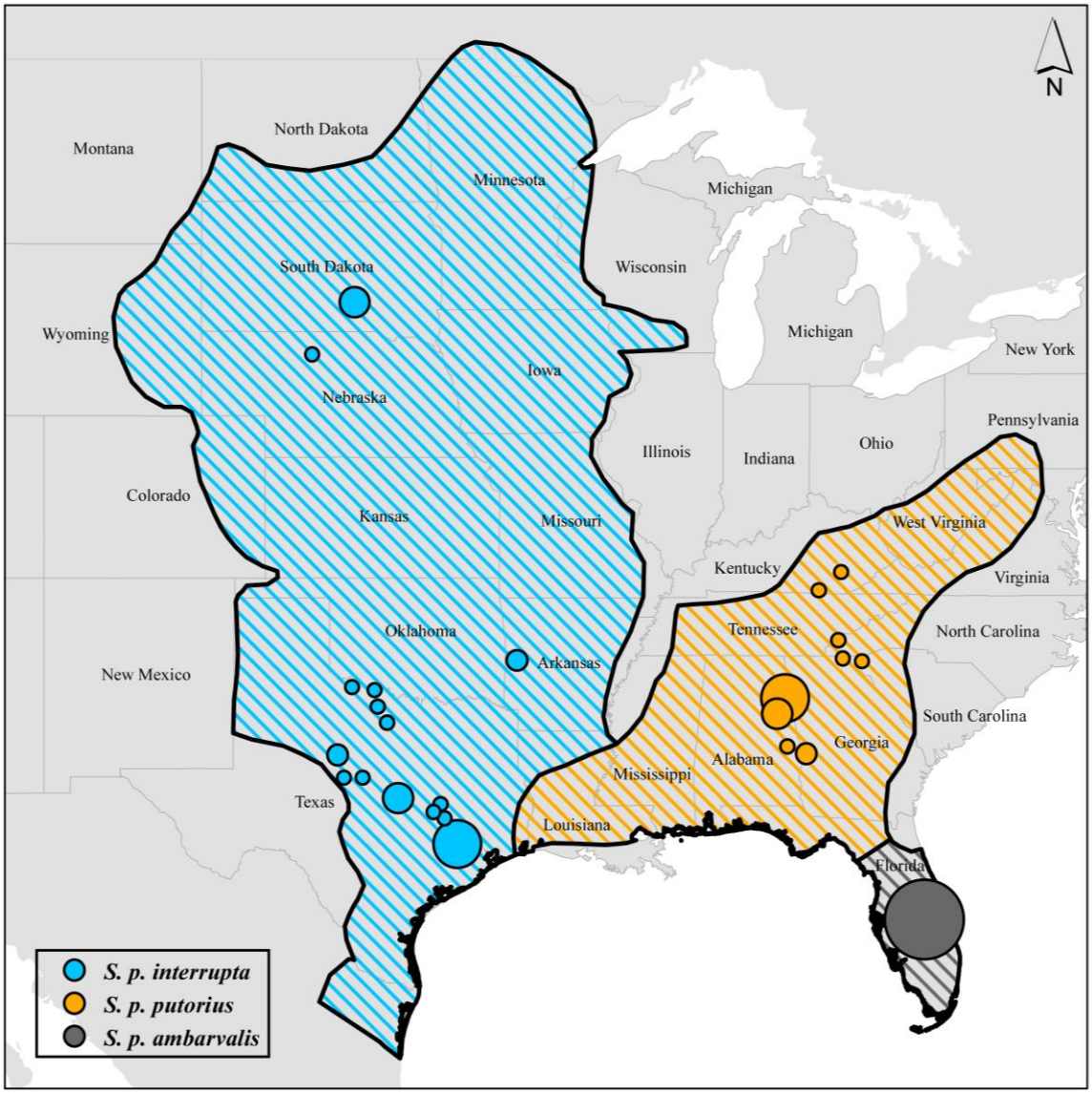


Table 2.—Non-vouchered eastern spotted skunk specimens examined for this study including the tissue type genomic DNA was extracted from, the morphological subspecies identification, sex, collection information, and the contact who facilitated the donation, loan, or field-acquired tissue. Acronyms for the collecting organizations are as follows: ASNHC or ASK (Angelo State Natural History Collection), AGFC (Arkansas Game and Fish Commission), FWC (Florida Fish and Wildlife Conservation Commission), and UWG (University of West Georgia). Tissue abbreviations are as follows: E (Ear clip), Hf (Hair follicle), L (Liver), Sk (Skin). NA = Not available, U = Unknown.

Tissue no.	Organization	Tissue type	Subspecies	Sex	State	County	Locality	Collection date	Latitude	Longitude	Contact
ASK10925*	ASNHC	E	*interrupta*	M	TX	Waller	NA	8-Oct-2015	NA	NA	R. Dowler
ASK10926*	ASNHC	E	*interrupta*	M	TX	Waller	NA	10-Oct-2015	NA	NA	R. Dowler
ASK11910	UWG	Sk	*putorius*	U	GA	Marion	Box Springs	17-Mar-2017	32.51259	-84.60673	A. Edelman
ASK11912	AGFC	Sk	*interrupta*	U	AR	Scott	Forest Service Road 507A	January 2016	34.69949	-94.28306	B. Sasse
ASK11913	ASNHC	Sk	*interrupta*	U	TX	Wilbarger	Vernon, Hwy 70	14-Mar-2016	34.15931	-99.27255	R. Dowler
ASK12480*	ASNHC	E	*interrupta*	M	TX	Harris	Hockley, Katy Prairie Conservancy	31-Oct-2016	29.94151	-95.84715	R. Dowler
ASK12482*	ASNHC	E	*interrupta*	M	TX	Harris	Hockley, Katy Prairie Conservancy	5-Nov-2016	29.95334	-95.85590	R. Dowler
ASK12490*	ASNHC	E	*interrupta*	M	TX	Harris	Hockley, Katy Prairie Conservancy	1-Nov-2016	29.94151	-95.84715	R. Dowler
ASK12693*	ASNHC	E	*interrupta*	M	TX	Coryell	Fort Hood	16-Dec-2016	31.31773	-97.82627	R. Dowler
UWG215	UWG	E	*putorius*	M	AL	Cleburne	Talladega National Forest	25-Feb-2015	NA	NA	A. Edelman
UWG305	UWG	E	*putorius*	M	AL	Clay	Cheaha State Park	8-Jul-2016	NA	NA	A. Edelman
UWG308	UWG	E	*putorius*	M	AL	Clay	Cheaha State Park	23-Jul-2016	NA	NA	A. Edelman
UWG355	UWG	E	*putorius*	F	AL	Cleburne	Talladega National Forest	17-Jan-2015	NA	NA	A. Edelman
UWG389	UWG	E	*putorius*	M	AL	Clay	Cheaha State Park	31-Jul-2016	NA	NA	A. Edelman
UWG424	UWG	E	*putorius*	M	AL	Clay	Cheaha State Park	3-Sep-2016	NA	NA	A. Edelman

Table 2.—Continued

Tissue no.	Organization	Tissue type	Subspecies	Sex	State	County	Locality	Collection date	Latitude	Longitude	Contact
UWG525	UWG	E	*putorius*	M	AL	Cleburne	Talladega National Forest	4-Apr-2015	NA	NA	A. Edelman
UWG585	UWG	E	*putorius*	M	AL	Cleburne	Talladega National Forest	4-Apr-2015	NA	NA	A. Edelman
UWG615	UWG	E	*putorius*	M	AL	Cleburne	Talladega National Forest	17-Jan-2015	NA	NA	A. Edelman
UWG645	UWG	E	*putorius*	M	AL	Cleburne	Talladega National Forest	17-Jan-2015	NA	NA	A. Edelman
UWG695	UWG	E	*putorius*	M	AL	Cleburne	Talladega National Forest	4-Apr-2015	NA	NA	A. Edelman
UWG865	UWG	E	*putorius*	M	AL	Cleburne	Talladega National Forest	30-Apr-2015	NA	NA	A. Edelman
FWC02	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	7-Oct-2015	27.81795	-81.13020	T. Hannon
FWC06	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	14-Oct-2015	27.84350	-81.15795	T. Hannon
FWC12	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	10-Nov-2015	27.81822	-81.12888	T. Hannon
FWC14	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	10-Nov-2015	27.82960	-81.13590	T. Hannon
FWC15	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	12-Nov-2015	27.80988	-81.12825	T. Hannon
FWC16	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	13-Nov-2015	27.81822	-81.12888	T. Hannon
FWC17	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	13-Nov-2015	27.81748	-81.12878	T. Hannon
FWC18	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	17-Nov-2015	27.81250	-81.13583	T. Hannon
FWC19	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	17-Nov-2015	27.81250	-81.13583	T. Hannon
FWC20	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	20-Nov-2015	27.82080	-81.14027	T. Hannon
FWC22	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	2-Feb-2016	27.86983	-81.14983	T. Hannon
FWC24	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	3-Feb-2016	27.86940	-81.15564	T. Hannon

Table 2.—Continued

Tissue no.	Organization	Tissue type	Subspecies	Sex	State	County	Locality	Collection date	Latitude	Longitude	Contact
FWC26	FWC	Hf	*ambarvalis*	M	FL	Osceola	Three Lakes Wildlife Management Area	16-Mar-2016	27.86590	-81.14921	T. Hannon
FWC27	FWC	Hf	*ambarvalis*	F	FL	Osceola	Three Lakes Wildlife Management Area	16-Mar-2016	27.86577	-81.14512	T. Hannon
FWC28	FWC	Hf	*ambarvalis*	M	FL	Osceola	Three Lakes Wildlife Management Area	17-Mar-2016	27.86571	-81.14551	T. Hannon
FWC29	FWC	Hf	*ambarvalis*	U	FL	Osceola	Three Lakes Wildlife Management Area	May 2016	U	U	T. Hannon
FWC30	FWC	Hf	*ambarvalis*	F	FL	Osceola	Three Lakes Wildlife Management Area	28-Mar-2016	27.81549	-81.14680	T. Hannon
FWC32	FWC	Hf	*ambarvalis*	M	FL	Osceola	Three Lakes Wildlife Management Area	28-Mar-2016	27.81478	-81.13156	T. Hannon
FWC40	FWC	Hf	*ambarvalis*	F	FL	Osceola	Three Lakes Wildlife Management Area	1-Apr-2016	27.81027	-81.13420	T. Hannon
FWC41	FWC	Hf	*ambarvalis*	F	FL	Osceola	Three Lakes Wildlife Management Area	25-May-2016	27.86919	-81.13655	T. Hannon
FWC42	FWC	Hf	*ambarvalis*	F	FL	Osceola	Three Lakes Wildlife Management Area	25-May-2016	27.86918	-81.15434	T. Hannon
FWC49	FWC	Hf	*ambarvalis*	M	FL	Osceola	Three Lakes Wildlife Management Area	16-Aug-2016	27.86539	-81.16206	T. Hannon
FWC50	FWC	Hf	*ambarvalis*	F	FL	Osceola	Three Lakes Wildlife Management Area	17-Aug-2016	27.86913	-81.15179	T. Hannon
FWC57	FWC	Hf	*ambarvalis*	F	FL	Osceola	Three Lakes Wildlife Management Area	19-Aug-2016	27.86537	-81.14930	T. Hannon

*Indicates samples obtained during field research for this study

in the form of dried ear clips or hair samples. Ear clips were frozen at -80˚C once received, while hair samples remained stored at room temperature in an air-tight container containing silica desiccant.

Laboratory methods.—Genomic DNA from non-hair samples was extracted using the QIAGEN DNeasy Blood & Tissue kit (QIAGEN Inc., Valencia, CA) following the manufacturer's protocol. DNA from hair follicles was extracted using the QIAGEN kit following the modifications outlined in Iudica et al. (2001) or with InstaGene matrix (Bio-Rad Inc., Hercules, CA) following the Chelex protocols of Suenaga and Nakamura (2005), with the exception that 10 hairs, instead of 2, were utilized per extraction. All DNA extracts were quantified on a Qubit 1.0 fluorometer (Invitrogen Corp., Carlsbad, CA). A total of 16 cross-species microsatellite loci were amplified using primers originally developed for closely-related mephitids and mustelids (Table 3; Bijlsma et al. 2000; Dragoo et al. 2009; Munguia-Vega et al. 2009; Floyd et al. 2011). Polymerase chain reaction (PCR) amplifications were performed in 10 or 25 μL reactions, for non-hair and hair samples, respectively. PCR reaction and cycling conditions were modified from the original primer publications and were optimized for the analysis of eastern spotted skunks in this study (Table 4). Reactions contained 5–50 ng DNA, 0.25 μM forward dye-labeled primer (Sigma-Aldrich Corp., St. Louis, MO), 0.25 μM reverse primer (Alpha DNA, Montreal, Quebec), 1.5 mM $MgCl_2$, 0.80 mM dNTPs (0.20 mM each; Thermo Fisher Scientific Inc., Waltham, MA), 1X Standard *Taq* Reaction Buffer (New England BioLabs Inc., Ipswich, MA), 0.4 U *Taq* DNA Polymerase (New England BioLabs Inc.), and de-ionized water as necessary to meet final reaction volumes. The only exception was for locus Meph42-25, whereby $MgCl_2$ was

Table 3.—Characterization of microsatellite loci optimized for genetic analysis of the eastern spotted skunk. Locus name, forward and reverse primer sequence, locus repeat motif, PCR annealing temperature (T_A, ˚C), average number of alleles per locus (N_A), allelic size range (bp), observed (H_O) and expected (H_E) heterozygosities, and the original publication of each primer are noted. All forward primers were dye-labeled. "—" = Not determined.

Locus	Primer sequence 5'-3'	Repeat motif	T_A	N_A	Size range	H_O	H_E	Reference
Meme5	F: CCTGAATGCAGGAGATGGAT R: GATGACTGATTAAAGCAGTCTGCC	$(CA)_{26}$	55	4.33	176–198	0.602	0.590	Munguia-Vega et al. 2009
Meme20	F: CATGAGCCCTGACAGGTGTA R: TCTTGGAACACTGCATCAAAA	$(GT)_{29}$	55	1.33	120–135	0.037	0.158	Munguia-Vega et al. 2009
Meme75	F: GTGTAGCTCTTCAGAGATGGATAGG R: TTCCAGGATGAACCAGGATG	$(GT)_{22}$	55	5.00	146–178	0.509	0.523	Munguia-Vega et al. 2009
Meme77[a]	F: TCCACAATAGTCAAACAATGGAA R: GTTGCAAATGGCAGGATTTT	$(CA)_{21}$	55	1.00	131–131	—	—	Munguia-Vega et al. 2009
Meme82[a]	F: TACCCGCTAGTTCCATCCAC R: GAGCCTATATGCCCATCAACA	$(CA)_{15}$	55	1.00	132–132	—	—	Munguia-Vega et al. 2009
Meme84[a]	F: GCAAAGGATATATTTGATAAGGGATT R: AATGGCTTTGTTTCCAGCAG	$(CA)_{15}$	55	1.00	139–139	—	—	Munguia-Vega et al. 2009
Meme88[a]	F: TAGCAGCAATGCCCACAATA R: CATTCTTTCTGATGGCTGCAT	$(CA)_{24}$	55	1.00	122–122	—	—	Munguia-Vega et al. 2009
Meph22-14	F: CTTTTGGGTCATTAGTGCATTTATG R: GGAAAGAGGAAAGAAAACCCATG	$(GT)_{24}$	50	3.67	230–256	0.358	0.337	Dragoo et al. 2009
Meph22-16*	F: GATCCCCCAAAACACAAAAACTATG R: GCTGGATAGCGCTGGCATG	$(GT)_{17}$	52	3.00	318–328	0.251	0.379	Dragoo et al. 2009
Meph22-26	F: ATGCAGGCTTGATTTTCAACCTC R: GAAAATCATTTACCAGTGGTGTGG	$(TG)_7(CG)_2(TG)_{12}$	52	7.67	220–240	0.783	0.805	Dragoo et al. 2009
Meph22-70	F: CAGATGCATCAGCAACGATTC R:GAGTGTTGCATTCAGCCTGTG	$(CA)_{24}$	50	11.33	185–217	0.740	0.820	Dragoo et al. 2009

Table 3.—Continued

Locus	Primer sequence 5'-3'	Repeat motif	T_A	N_A	Size range	H_O	H_E	Reference
Meph22-89[a]	F: GGCTCATATTCCCCTGGGTAGG R: TGAAAGGGGGTGGAAGAACTGG	$(TG)_6(AG)(TG)_{13}$	56	1.00	195–195	—	—	Dragoo et al. 2009
Meph42-25	F: ACCACTGTTGCACCAGTTCTATCAG R: CACAGTTAGAAGGCCCAGAACATTC	$(TG)_{24}$	54	7.00	201–236	0.688	0.732	Dragoo et al. 2009
Meph42-73	F: AAAGGACAATCCCACAGGTCT R: TGGACATGGAATTCTGGTTG	$(CA)_{14}$	50	6.00	152–168	0.685	0.688	Dragoo et al. 2009
Mel 1	F: CTGGGGAAAATGGCTAAACC R: AATGCAGGCTTTGCAATTCC	$(GT)_{20}$	60	6.33	258–278	0.709	0.743	Bijlsma et al. 2000
nRIO-08[b]	F: TGAGGTGTTGGTGTTTTGTTCTAT R: TTGCCTGCTGACATTGAAGMT	$(TG)_{15}$	58	4.33	139–159	0.394	0.432	Floyd et al. 2011

[a]Indicates monomorphic loci excluded from final genetic analysis

[b]Primer originally published by Beheler et al. (2004); annealing sequence modified for use in *Spilogale* by Floyd et al. (2011)

*Deviates significantly from Hardy-Weinberg equilibrium ($P_{adj} < 0.05$)

Table 4.—Polymerase chain reaction thermal profiles utilized at each microsatellite locus for genetic analysis of the eastern spotted skunk. Locus specific annealing temperatures (T_A) are provided in Table 3.

Thermal profile	Loci
95˚C for 3min, followed by 35 cycles of 95˚C for 30s, T_A for 30s, and 72˚C for 1min, with a final extension at 72˚C for 10min	Meph22-14, Meph22-16, Meph22-26, Meph22-70, Meph22-89, Meph42-25, Meph42-73
94˚C for 5min, followed by 40 cycles of 94˚C for 30s, 55˚C for 30s, and 72˚C for 30s, with a final extension at 72˚C for 5min	Meme5, Meme20, Meme75, Meme77, Meme82, Meme84, Meme88
94˚C for 3min, followed by 30 cycles of 94˚C for 1min, 60˚C for 2min, and 72˚C for 1.5min, with a final extension at 72˚C for 10min	Mel1
94˚C for 4min, followed by 40 cycles of 94˚C for 40s, 58˚C for 40s, and 72˚C for 1min, with a final extension at 72˚C for 5min	nRIO-08

increased to 3 mM. Dye-labeled PCR products were genotyped on a capillary electrophoretic genetic analysis system (CEQ™8000, Beckman-Coulter Inc., Brea, CA) utilizing the 400 bp GenomeLab DNA Size Standard Kit (AB Sciex, Concord, Ontario) as the size standard. Genotypes were scored by eye. To mitigate the presence of scoring errors in the final dataset, and to reduce their negative effects in downstream analyses, 26% of tissue samples and 35% of hair samples were reamplified and analyzed, in addition to approximately 20% of all samples analyzed being scored more than once to ensure consistent genotype calls (DeWoody et al. 2006).

Analysis of microsatellite variation. —FreeNA was used to determine the frequency of null alleles for all loci and populations in the dataset (Chapuis and Estoup 2007). Scoring errors due to stutter and large-allele dropout were assessed with Micro-Checker v 2.2.3 (van Oosterhout et al. 2004). Tests for Hardy-Weinberg equilibrium and genotypic disequilibrium between loci were conducted using the Markov chain approximation (dememorization: 10,000; batches: 1,000; iterations per batch: 10,000) in GENEPOP v 4.5.1 (Raymond and Rousset 1995). *P*-values were adjusted for multiple pairwise comparisons using a Bonferroni correction in R (R Core Team 2016). GenAlEx v 6.5 (Peakall and Smouse 2006, 2012) was used to assess levels of genetic variation including the number of alleles per locus (N_A), observed (H_O) and expected heterozygosities (H_E), and the number of private alleles (N_P) within each population. Differences in genetic diversity among subspecies were determined using randomized t-tests in R (R Core Team 2016) and resulting *P*-values were adjusted using a Bonferroni correction.

Analysis of genetic structure.—The program STRUCTURE v 2.3.4 (Pritchard et al. 2000) was used to determine the presence of genetic clusters within the eastern spotted

skunk. Using the admixture model and correlated allele frequencies, 20 independent runs were performed at each assumed population number (K = 1–10). No putative population origin information was provided *a priori*. The length of the burn-in period and number of Markov chain Monte Carlo iterations post-burn-in were set to 50,000 and 200,000, respectively. To determine the optimum number of population clusters present, ΔK was calculated using STRUCTURE HARVESTER v 0.6.94 (Earl and vonHoldt 2012), following the recommendation by Evanno et al. (2005). CLUMPP v 1.1.2 was used to average individual membership coefficients from the 20 replicate STRUCTURE runs at the specified ΔK using the FullSearch algorithm (Jakobsson and Rosenberg 2007). Finally, we used the program STRUCTURE PLOT v 2 (Ramasamy et al. 2014) to generate graphical displays of individual membership.

To further examine the presence of genetic structure, a principal coordinates analysis (PCoA) was conducted in GenAlEx v 6.5 (Peakall and Smouse 2006, 2012), with the input being a distance table (Smouse and Peakall 1999) generated from the final genotypic data. A permutational multivariate analysis of variance (PERMANOVA; n = 9,999 permutations) was used to determine the significance of the PCoA clusters using the adonis function within the vegan package (Oksanen et al. 2017) in R (R Core Team 2016). The degree of genetic differentiation among subspecies was assessed by calculating pairwise F_{ST} values using the *ENA* (excluding null alleles) correction method by Chapuis and Estoup (2007) in FreeNA. Null alleles, or the non-amplification of alleles due to sources such as mutation in the flanking region (primer sequence) or low-quality DNA templates, can positively bias F_{ST} values, as they generally function to reduce within-population diversity. The correction method implemented by FreeNA (Chapuis and Estoup 2007) has been shown to effectively

correct for this positive bias that could result in the presence of null alleles. Rates of gene flow among subspecies were determined from *Nm*, the product of the effective population sizes (*N*) and the rate of migration (*m*) between them, using Wright's (1984) estimator: $Nm = (1 / F_{ST} - 1) / 4$. Additionally, 2-way Mantel tests were performed in R (R Core Team 2016) using the package ade4 (Dray and Dufour 2007) to determine if genetic isolation by distance (IBD) was present within each subspecies.

RESULTS

Sample collection. —Field efforts led to the live capture of 6 plains spotted skunks from Coryell (n = 1), Harris (n = 3), and Waller (n = 2) counties in Texas. All other acquired samples were obtained through tissue loans or donations (n = 63), by salvaging road-killed animals (n = 7), or from fur trapper harvests (n = 5). In total, this study included 81 individuals representing all 3 subspecies: the plains spotted skunk (n = 36), the Appalachian spotted skunk (n = 21), and the Florida spotted skunk (n = 24). Although hair samples from non-permanently marked individuals represented the entire Florida spotted skunk sample, all proved to be unique individuals.

Microsatellite variation. —Of the 16 cross-species microsatellite loci genotyped, 2 proved monomorphic for all 3 subspecies (Meme82 and Meme84) and were excluded from further analyses. An additional 3 loci (Meme77, Meme88, and Meph22-89) were monomorphic within the plains and Appalachian subspecies. Due to the limited quantity of DNA from all Florida spotted skunks, only a few individuals representing a random subset were amplified across these 3 loci, in supposition that they would also prove monomorphic and uninformative for this study. This subset also proved to be monomorphic for the same allele, therefore no further individuals were analyzed at these loci. In all, with the exclusion of those 5 loci, the final genotypic dataset contained 81 individuals analyzed across 11 microsatellite loci (Appendix 1). The genotyping error rate for non-hair samples was 1.52% and 2.34% for hair samples. All but 1 genotyping error was attributed to a single locus, Meph22-16, for hair samples. These error percentages are unlikely to affect conclusions relating to genetic diversity or structure, as it has been shown that estimates of H_E, F_{ST}, and structure remain unbiased, even in the presence of high (>30%) genotyping error rates, when

n >10 individuals per population (Smith and Wang 2014). The entire dataset contained 0.79% missing data, well below the maximum 20% suggested by Smith and Wang (2014) for the purposes of accurately examining population genetics.

Two microsatellite loci analyzed served to distinguish eastern spotted skunk subspecies. Locus Meme20 was perhaps the least informative marker with respect to its allelic richness (N_A = 1.33) and heterozygosity levels (H_O range: 0.037–0.158); however, it differentiated the plains subspecies from both the Appalachian and Florida subspecies due to its monomorphic nature in the latter and polymorphic nature in the former. Conversely, locus Meph22-14 was monomorphic within the plains subspecies, yet was highly polymorphic within the Appalachian and Florida subspecies. Additionally, a unique pattern emerged within locus Meph42-25 (dinucleotide repeat), as all alleles ranging from 201–215 base pairs (bp) were odd-numbered fragment sizes, yet alleles from 218–236 bp were even-numbered sizes. However, this pattern did not serve as a diagnostic character to differentiate subspecies, as all 3 contained bp fragments within the range of 201–236.

Null allele frequencies greater than 10% were present within *S. p. interrupta* at locus Meme20 (25.0%) and within *S. p. putorius* at locus Meph22-16 (11.3%). Across all loci and subspecies, the null allele frequency averaged 3.51 ± 0.009% ($\bar{X}$ ± SE). Scoring errors due to stutter might have affected genotyping of the plains subspecies at locus Meph22-26, while evidence of scoring errors due to large-allele dropout were not detected within any subspecies or at any locus. Because evidence of scoring errors due to stutter and high null allele frequencies were not consistently detected at specific loci across subspecies, these loci were retained in further analyses. Across loci, N_A for the 3 subspecies ranged from 4.73–6.55, while H_O and H_E ranged from 0.449–0.623 and 0.485–0.627, respectively (Table 5). For all

Table 5.—Genetic diversity values for each eastern spotted skunk subspecies across 11 microsatellite loci. N_A is mean number of alleles per locus, H_O is observed heterozygosity, H_E is expected heterozygosity, and N_P is the number of private alleles. Values for N_A, H_O, and H_E are mean ± SE with ranges provided in parentheses.

Subspecies	*n*	N_A	H_O	H_E	N_P
S. p. interrupta	36	6.55 ± 1.201 (2–15)	0.498 ± 0.091 (0.028–0.806)	0.581 ± 0.090 (0.027–0.893)	29
S. p. putorius	21	4.73 ± 0.764 (1–10)	0.449 ± 0.073 (0.000–0.762)	0.485 ± 0.078 (0.000–0.840)	9
S. p. ambarvalis	24	5.09 ± 0.707 (1–9)	0.623 ± 0.083 (0.000–0.905)	0.627 ± 0.079 (0.000–0.859)	8
Average	27	5.46 ± 0.531	0.523 ± 0.048	0.564 ± 0.047	15.3

loci and subspecies, N_A was 5.46 ± 0.531 and H_O was 0.523 ± 0.048 ($\bar{X}$ ± SE; Table 5). Genetic diversity, with respect to N_A, H_O, and H_E, was not significantly different among the 3 subspecies (randomized t-test; n = 10,000 iterations; $P_{adj} > 0.64$ for all comparisons). Private alleles, or alleles present only within a single population (in this case, subspecies) were approximately 3 times more abundant within the plains spotted skunk in comparison to the Appalachian or Florida spotted skunks (Table 5).

Significant departure from Hardy-Weinberg equilibrium was present at locus Meme20 within the plains subspecies only ($P_{adj} < 0.0001$). When all 3 subspecies were pooled, the only deviation from Hardy-Weinberg equilibrium occurred at locus Meph22-16 ($P_{adj} = 0.015$; Table 3). These loci were included in all further analyses due to their deviations not being consistently encountered across subspecies. Hardy-Weinberg equilibrium could not be determined for locus Meme20 when subspecies were pooled or for *S. p. putorius* and *S. p. ambarvalis* individually, as these 2 subspecies were monomorphic at this locus. Linkage disequilibrium was detected between loci Meme5 and Meph22-70 within the Florida subspecies ($P_{adj} = 0.007$) and when subspecies were pooled ($P_{adj} = 0.009$).

Genetic structure.—Tests of genetic structure including all 3 subspecies resulted in $\Delta K = 2$ (Fig. 2A). All individuals belonging to *S. p. interrupta* formed a single cluster, while all individuals belonging to both *S. p. putorius* and *S. p. ambarvalis* comprised a second cluster (Fig. 2A). Because STRUCTURE identifies clusters corresponding to the uppermost hierarchical level of structure present (Evanno et al. 2005), a second STRUCTURE analysis was performed that excluded the plains subspecies to determine if a lower level of hierarchical structure was present between the Appalachian and Florida subspecies. The analysis excluding the plains subspecies resulted in $\Delta K = 2$, with *S. p. putorius* individuals

Fig. 2.—Plots of Δ*K* for *K* = 1–10 from STRUCTURE HARVESTER and the respective STRUCTURE PLOT bar graphs for *Spilogale putorius interrupta*, *S. p. putorius*, and *S. p. ambarvalis* (A) and *S. p. putorius* and *S. p. ambarvalis* (B), indicating Δ*K* = 2 for both analyses. For bar graphs, each bar represents 1 individual and its respective membership coefficient when *K* = 2.

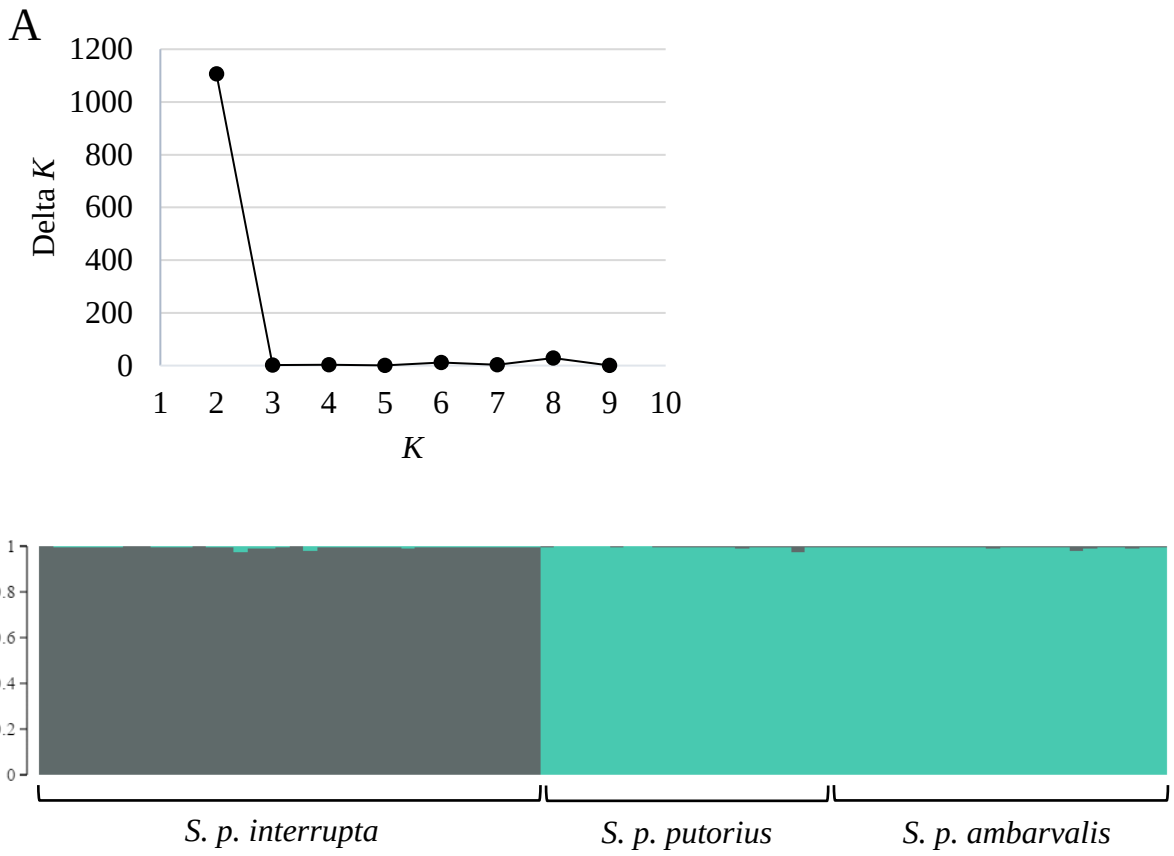
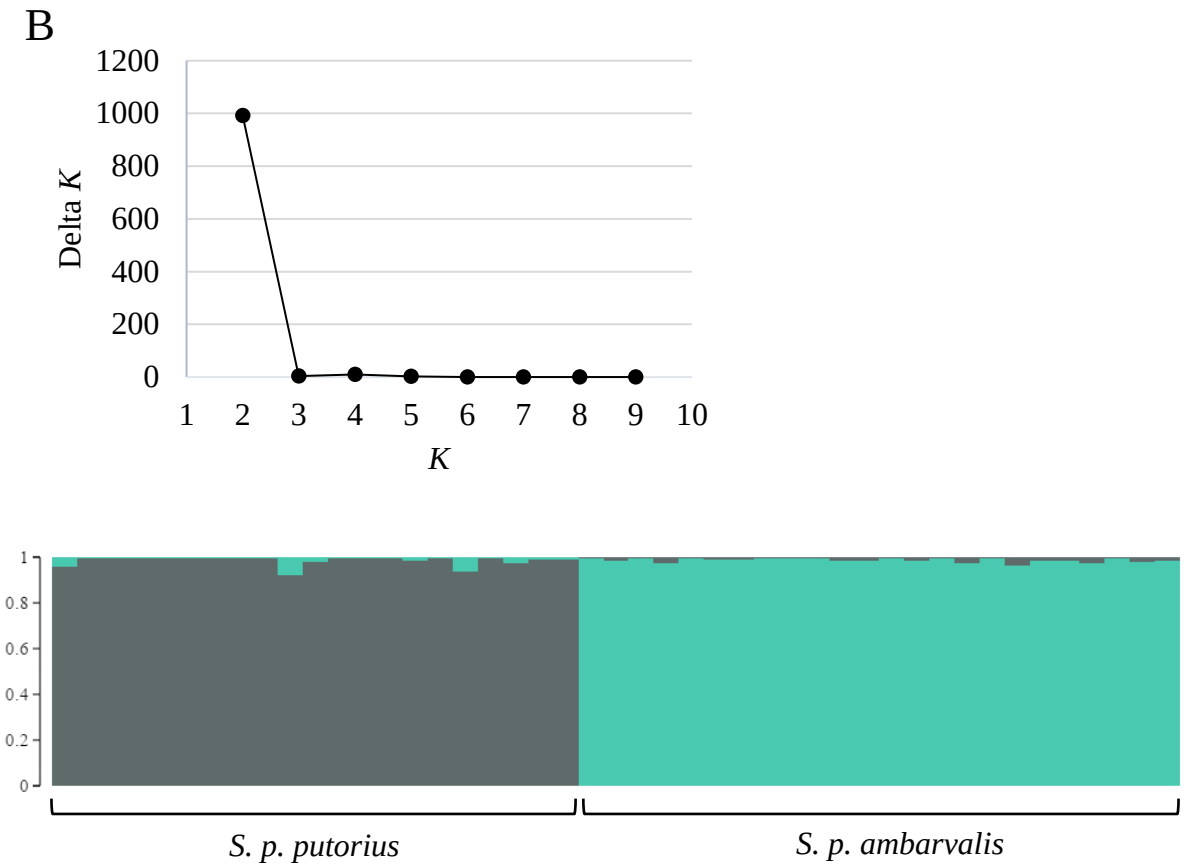


Fig. 2.—Continued



and *S. p. ambarvalis* individuals forming separate clusters (Fig. 2B). STRUCTURE plots indicated a very low degree of admixture among subspecies (Fig. 2A, 2B). In addition, average membership coefficients were high for individuals within their respective subspecies and averaged within subspecies ($\bar{X} \pm SE$): *S. p. interrupta* (99.49 ± 0.001%), *S. p. putorius* (98.39 ± 0.004%), and *S. p. ambarvalis* (98.70 ± 0.002%).

The PCoA analysis further supported the presence of genetic structure among the 3 subspecies. The first axis, explaining 24.70% of the variation present, separated the plains subspecies from both the Appalachian and Florida subspecies (Fig. 3). The 2nd axis, explaining 10.28% of the variation present, separated the Appalachian and Florida subspecies and subdivided individuals within the plains subspecies, yet no geographical significance could be drawn from this subdivision (Fig. 3). Additionally, the PERMANOVA supported statistical significance of subspecies groupings ($F = 45.10$, $P < 0.0001$). One individual (ASK7931, Waller Co., TX) within the plains subspecies appeared intermediate between the plains and Florida subspecies for PCoA, yet its average membership to *S. p. interrupta* was 99.03%.

Corrected estimates of pairwise F_{ST} among subspecies ranged from 0.195 to 0.338 (Table 6), with the highest degree of genetic differentiation occurring between the plains and Appalachian subspecies ($F_{ST} = 0.338$), and the lowest degree occurring between the Appalachian and Florida subspecies ($F_{ST} = 0.195$). Uncorrected estimates of F_{ST} (range: 0.204–0.339) were similar to the *ENA* corrected estimates, but were inflated slightly, likely due to the presence of null alleles. Rates of gene flow among subspecies were low (*Nm* range: 0.490–1.032), most notably between the plains and Appalachian subspecies (*Nm* =

Fig. 3.—Results from the principal coordinates analysis of genotypes of 11 microsatellite loci for subspecies of *Spilogale putorius*. The first axis explained 24.70% of the variation in the data and separated the plains subspecies from the Appalachian and Florida subspecies, while the second axis explained 10.28% of the variation in the data and separated the Appalachian from the Florida subspecies. Individual ASK7931 appeared intermediate between *S. p. interrupta* and *S. p. ambarvalis*, yet its membership coefficient to *S. p. interrupta* was 99.03%.

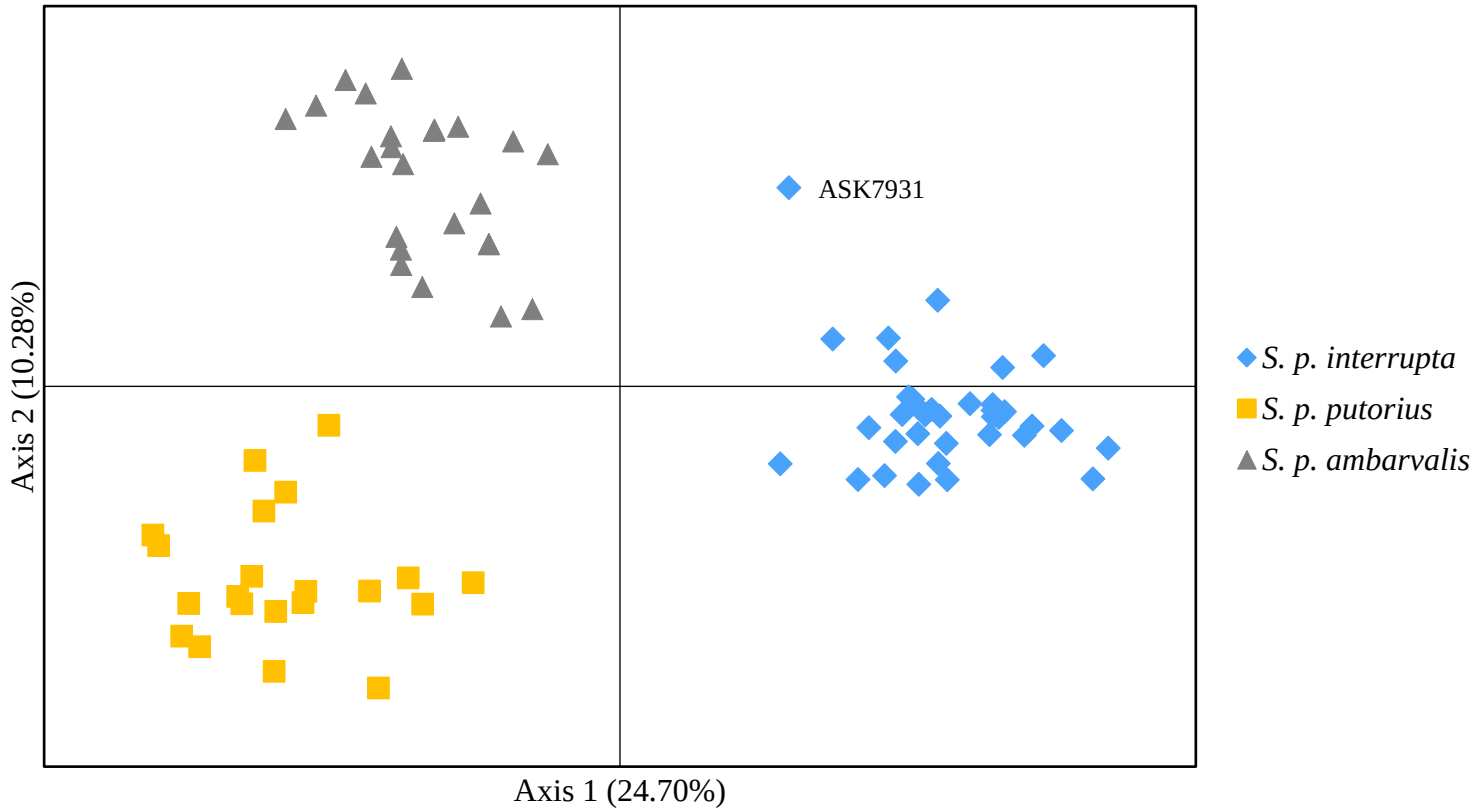


Table 6.—Degree of genetic differentiation (F_{ST}; below diagonal) and rate of gene flow (N_m, above diagonal) among eastern spotted skunk subspecies.

Subspecies	*S. p. interrupta*	*S. p. putorius*	*S. p. ambarvalis*
S. p. interrupta	—	0.490	0.738
S. p. putorius	0.338	—	1.032
S. p. ambarvalis	0.253	0.195	—

0.490; Table 6). An association between geographic and genetic distance was detected within the plains spotted skunk ($r = 0.24$; $P = 0.007$) and less strongly in the Florida spotted skunk ($r = 0.10$; $P = 0.045$).

DISCUSSION

Van Gelder (1959) initially identified *S. putorius* as a polytypic species composed of 15 subspecies, 3 of which are still recognized today (*S. p. interrupta*, *S. p. putorius*, *S. p. ambarvalis*). Results from our genetic structure analyses indicated the presence of 3 genetic clusters commensurate with the 3 subspecies designations. Although Van Gelder (1959) only utilized variation in external measurements (i.e. total, tail, and hind foot length), color pattern, and locality to designate *S. putorius* subspecies, we now add microsatellite variability to this list. Evidence of genetic structure and differentiation within the eastern spotted skunk was present in all analyses; however, the inability of STRUCTURE to separate *S. p. putorius* from *S. p. ambarvalis* in the first analysis was likely due to the less pronounced differentiation observed between these subspecies in comparison to the high degree of differentiation the plains spotted skunk shared with both the Appalachian and Florida subspecies. This high degree of differentiation likely resulted in $\Delta K = 2$, instead of $\Delta K = 3$, when all 3 subspecies were analyzed together. Mean LnP(K) for $\Delta K = 3$ was less negative than $\Delta K = 2$ (-1179.79 vs. -1204.77); however, the *SD* of this value was higher for $\Delta K = 3$ (10.42 vs. 0.16).

Although IBD was present within the plains spotted skunk, the PCoA analysis did not reveal the same pattern, as individuals from Texas, South Dakota, Arkansas, and Nebraska formed a tight cluster with no discernable geographic pattern (Fig. 3). Within the Florida subspecies, the presence of IBD was relatively unexpected, as all individuals were sampled from 1 contiguous population within a small geographic area. The greatest distance separating 2 trapped Florida spotted skunks was only 3.88 km, with an average distance of 1.51 km. As seasonal home ranges for the eastern spotted skunk (which are highly sex and

season dependent) have been reported to range from 19–1,824 ha (Lesmeister et al. 2009), and the correlation coefficient for IBD was weak within this subspecies (r = 0.10), it is possible that IBD does not play a key role in the structure of this subspecies at the scale we sampled. Interestingly, despite all Florida spotted skunk samples deriving from a single population, this subspecies displayed a pattern of genetic variation similar to that observed in the plains and Appalachian subspecies, whose samples originated from as many as 5 states with a maximum distance of >1,500 km separating individuals.

A comparison of genetic diversity of the plains spotted skunk to other, and perhaps more common, North American mesocarnivores highlights the reduced diversity observed in this subspecies. Observed heterozygosity for the plains spotted skunk averaged 0.498, while H_O for subspecies of the North American badger (*Taxidea taxus*) averaged 0.757 (Kyle et al. 2004), studies on the striped skunk (*Mephitis mephitis*) reported H_O values of 0.764 and 0.683 (Barton and Wisely 2012; Brashear et al. 2015), and H_O for Florida populations of raccoons (*Procyon lotor*) ranged from 0.78 to 0.84. (Trujillo and Hoffman 2016). Allelic richness for the plains spotted skunk averaged 6.55, whereas N_A for *T. taxus* averaged 9.9 (Kyle et al. 2004), *M. mephitis* averaged 12.88 and 10.69 (Barton and Wisely 2012; Brashear et al. 2015), and *P. lotor* averaged 8.77 for mainland Florida populations (Trujillo and Hoffman 2016). Instead, average H_O and N_A of the plains subspecies more closely resembles the levels found within the island spotted skunk (H_O: 0.590, N_A: 4.5; Floyd et al. 2011), an insular subspecies of the western spotted skunk restricted to 2 islands within the Channel Island archipelago.

However, in contrast to the trend of lower genetic diversity observed in *S. p. interrupta* when compared to more common, North American carnivores, the plains

subspecies exhibits levels of genetic diversity higher than those reported for endangered carnivores. For example, grassland and shrubland (sub)species such as the San Joaquin kit fox (*Vulpes macrotis mutica*) and the black-footed ferret (*Mustela nigripes*) contain low measures of genetic diversity due to reductions in population connectivity as a result of habitat alteration. Schwartz et al. (2005) reported ranges of N_A and H_O for the San Joaquin kit fox at 2.65–4.38 and 0.28–0.50, respectively. Cain et al. (2011) determined $N_A = 2$ for 2 subpopulations of black-footed ferret with H_O ranging from 0.39–0.44. Other endangered carnivores such as the clouded leopard (*Neofelis nebulosa*) and Amur tiger (*Panthera tigris altaica*) exhibit the same trend of reduced genetic variability (Buckley-Beason et al. 2006; Henry et al. 2009). In a comparison across vertebrate taxa representing all 6 IUCN conservation ranks, Willoughby et al. (2015) determined that genetic diversity values (H_O and N_A) were lower in threatened vertebrates, which exhibit some degree of extinction risk, in comparison to species of lesser conservation concern. Given the vulnerable status of the eastern spotted skunk by the IUCN, and that the conservation status of the plains subspecies is currently under review, the lower-than-average genetic diversity observed within each subspecies agrees with the pattern evidenced by Willoughby et al. (2015).

Levels of genetic diversity did not significantly differ among the 3 subspecies, therefore suggesting that the plains spotted skunk is no more depauperate genetically than the Appalachian or Florida spotted skunks. However, trends in sightings and capture rates for the 3 subspecies are not equal, suggesting relative abundances vary by subspecies. For example, past studies have reported that *S. p. ambarvalis* is abundant in southern (Kaplan and Mead 1991) and east-central (Kinlaw et al. 1995) Florida, and the recent trapping success rate by the Florida Fish and Wildlife Conservation Commission was substantially greater

(approximately 42%; Tina Hannon, personal communication, June 2017) than those obtained in recent literature. A recent publication reporting on incidental captures of the Appalachian spotted skunk (n = 6 over a month period; Diggins et al. 2015) and the number of *S. p. putorius* tissue donations received for this analysis (n = 15) suggest that this subspecies is more locally abundant than the plains subspecies. For the plains spotted skunk, studies to date have reported capture rates of 0.38% (Hackett et al. 2007) and 0.17% (this study), and game camera detections of 2 or 3 individuals over a period of 26 months (Hardy 2013), thus highlighting the rarity of this subspecies throughout its range in comparison to the other 2 subspecies.

Recent phylogeographic work by Ferguson et al. (2017) revealed that the genus *Spilogale* diverged from other mephitid lineages approximately 6.53 Ma, with eastern and western spotted skunks sharing a most recent common ancestor 2.71 Ma. Because it follows that differentiation achieved within *S. putorius* occurred after its divergence from *S. gracilis*, the intraspecies divergence observed likely occurred throughout the Quaternary, as opposed to occurring pre-Pleistocene. Biological communities in North America were affected continent-wide due to river system modifications, sea level changes, lake creation, and climate cooling that occurred as a result of alternating glacial and interglacial periods. Several geographic barriers to gene flow likely functioned to create the patterns of genetic differentiation and structure presently observed among eastern spotted skunk subspecies. The eastern spotted skunk is certainly not the only species that displays these patterns, as genetic signatures of isolation are abundant in the literature due to climatic and geological changes that occurred within the Quaternary (Hayes and Harrison 1992; Barton and Wisely 2012; Ferguson et al. 2017). Specifically, during the Wisconsinan glaciation of the Pleistocene, the

southern United States and Mexico served as refugia for spotted skunks (Van Gelder 1959; Ferguson et al. 2017). Eventual retreat of the ice sheets enabled present day eastern spotted skunks to extend their range both northward and eastward, with individuals eventually branching east and west of the southern Mississippi River (Van Gelder 1959). Not only did this river serve as a strong isolating barrier, especially during periods of interglacial melt when river volume and width were substantial, but the floodplains and moist lowlands along the river also provided unsuitable habitat for eastern spotted skunks, further restricting gene flow across its banks (Van Gelder 1959). This divergence at the Mississippi River is congruent with the current subspecies boundary between *S. p. interrupta* and *S. p. putorius*, and is a well-documented barrier to gene flow in a variety of taxa (Burbrink et al. 2000; Brant and Ortí 2003; Soltis et al. 2006; Brandley et al. 2010; Near et al. 2001).

The divergence of the Florida spotted skunk from the plains and Appalachian subspecies is less clear, yet fossils indicate the earliest colonization of Florida by spotted skunks occurred in the early Pleistocene (Webb 1974). Climatic and glacial fluctuations that occurred throughout the Quaternary altered sea levels, with evidence for much of Florida being inundated over several periods from 188,000 to 72,000 BP (Cronin et al. 1981). This marine barrier likely served to isolate Florida populations of spotted skunks, much like it has in other species, such as the woodrat (*Neotoma* spp.; Hayes and Harrison 1992). With the recession of sea levels into the Holocene and the alleviation of the marine barrier, Florida populations could then achieve secondary contact with present day *S. p. putorius*. However, few to no specimens are documented or contained within museum collections along this subspecies contact zone, therefore making it difficult to interpret the degree of introgression that occurs. Moreover, much remains unknown with respect to the timing of genetic

divergences among all 3 subspecies, thus future studies addressing the phylogeographic patterns of *S. putorius* are desperately needed.

Fluctuating climatic conditions during the Quaternary could have dictated the structure and differentiation present within this species; however, modern-day anthropogenic activity has great capacity to exacerbate this differentiation by reducing population sizes and genetic variability. The negative effects of habitat fragmentation on the genetic variability of numerous carnivore species are well documented; however, these impacts have yet to be determined for the eastern spotted skunk. Prevalent anthropogenic sources that have the potential to act as barriers to gene flow for the plains spotted skunk include gas and oil drilling practices, urban sprawl, and agricultural modification of the landscape. Specifically, fragmentation has been shown to reduce gene flow and genetic variability within impacted carnivore populations (Riley et al. 2006; Haag et al. 2010; Schwalm et al. 2014; McManus et al. 2015) and often leads to the implementation of conservation and management strategies for the affected species. Although structure below the subspecies level was not found in this analysis, thus indicating impediments to gene flow within subspecies are not present, the intensification of anthropogenic activities throughout the central United States have the potential to restrict gene flow in this region. Therefore, any future management strategies for the plains spotted skunk should account for the dynamic nature of this habitat.

Although the 11 cross-species microsatellite markers utilized in this study enabled an in-depth analysis of the genetic structure and differentiation within this species, the development of *Spilogale*-specific primers would aid in future studies of the eastern spotted skunk. From an initial set of 26 molecular markers we tested on *Spilogale* that were originally developed for the striped skunk (Dragoo et al. 2009; Munguia-Vega et al. 2009),

Eurasian badger (Bijlsma et al. 2000), and North American river otter (Beheler et al. 2004; Floyd et al. 2011), only 16 successfully amplified in *Spilogale*. Of these 16, 5 were monomorphic. As less than half of the loci tested were unsuccessfully amplified or proved uninformative for this study, the development of *Spilogale*-specific markers would not only enable the analysis of additional neutral sites, but would also ensure a higher prevalence of polymorphic loci for downstream analysis. Given that the conservation status of this species is insecure, markers specific for the eastern spotted skunk would be beneficial in addressing additional questions from individual (i.e. hybridization with the western spotted skunk) to population level scales. In addition, an analysis of several mitochondrial genes would help address the presence of more deeply rooted genetic divergences among subspecies and would enable a comparison of nuclear and mitochondrial evolutionary histories.

In conclusion, the eastern spotted skunk displays strong patterns of genetic structuring and differentiation among subspecies, which are commensurate with previously reported morphological differences (Van Gelder 1959). The presence of private alleles found in all 3 subspecies, the degree of differentiation among them, the lack of gene flow, and high individual membership coefficients indicate the need to consider each subspecies as a unique evolutionarily significant unit (Moritz 1994). A similar suggestion was provided by Floyd et al. (2011) for the island spotted skunk, as they determined that populations occupying 2 separate Channel Islands, Santa Cruz Island and Santa Barbara Island, were just as differentiated from each other as they were from mainland western spotted skunk subspecies. Future management strategies for the eastern spotted skunk should therefore consider the genetic dissimilarities present among subspecies, as it is possible that these genetic differences reflect behavioral, physiological, or habitat differences, as well. Although we

were able to sample a wide geographic range representing all 3 subspecies, the inclusion of specimens representing additional states would help determine the amount of genetic introgression occurring. Furthermore, the inclusion of additional individuals that occupy subspecies contact zones would help refine our understanding of the geographic barriers that acted and are currently serving to divide the subspecies.

LITERATURE CITED

BARTON, H. D., AND S. M. WISELY. 2012. Phylogeography of striped skunks (*Mephitis mephitis*) in North America: Pleistocene dispersal and contemporary population structure. Journal of Mammalogy 93:38-51.

BEHELER, A. S., J. A. FIKE, L. M. MURFITT, O. E. RHODES, JR., AND T. S. SERFASS. 2004. Development of polymorphic microsatellite loci for North American river otters (*Lontra canadensis*) and amplification in related mustelids. Molecular Ecology Notes 4:56-58.

BIJLSMA, R., M. VAN DE VLIET, C. PERTOLDI, R. C. VAN APELDOORN, AND L. VAN DE ZANDE. 2000. Microsatellite primers from the Eurasian badger, *Meles meles*. Molecular Ecology 9:2215-2216.

BOPPEL, P. J., AND C. A. LONG. 1994. Status of the spotted skunk (*Spilogale putorius*) in its northeastern range, north-central United States. Small Carnivore Conservation 11:11-12.

BRANDLEY, M. C., T. J. GUIHER, R. A. PYRON, C. T. WINNE, AND F. T. BURBRINK. 2010. Does dispersal across an aquatic geographic barrier obscure phylogeographic structure in the diamond-backed watersnake (*Nerodia rhombifer*)?. Molecular Phylogenetics and Evolution 57:552-560.

BRANT, S. V., AND G. ORTÍ. 2003. Phylogeography of the Northern short-tailed shrew, *Blarina brevicauda* (Insectivora:Soricidae): past fragmentation and postglacial recolonization. Molecular Ecology 12:1432-1449.

BRASHEAR, W. A., L. K. AMMERMAN, AND R. C. DOWLER. 2015. Short-distance dispersal and lack of genetic structure in an urban striped skunk population. Journal of Mammalogy 96:72-80.

BUCKLEY-BEASON, V., ET AL. 2006. Molecular evidence for species-level distinctions in clouded leopards. Current Biology 16:2371-2376.

BURBRINK, F. T., R. LAWSON, AND J. B. SLOWINSKI. 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution 54:2107-2118.

CAIN, C. M., T. M. LIVIERI, AND B. J. SWANSON. 2011. Genetic evaluation of a reintroduced population of black-footed ferrets (*Mustela nigripes*). Journal of Mammalogy 92:751-759.

CHAPUIS, M., AND A. ESTOUP. 2007. Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution 24:621-631

CHOATE, J. R., E. D. FLEHARTY, AND R. J. LITTLE. 1973. Status of the spotted skunk, *Spilogale putorius*, in Kansas. Transactions of the Kansas Academy of Science 76:226-233.

CRABB, W. D. 1948. The ecology and management of the prairie spotted skunk in Iowa. Ecological Monographs 18:201-232.

CRONIN, T. M., B. J. SZABO, T. A. AGER, J. E. HAZEL, AND J. P. OWENS. 1981. Quaternary climates and sea levels of the U.S. Atlantic Coastal Plain. Science 211:233-240.

DEWOODY, J., J. D. NASON, AND V. D. HIPKINS. 2006. Mitigating scoring errors in microsatellite data from wild populations. Molecular Ecology Notes 6:951-957.

DIGGINS, C. A., D. S. JACHOWSKI, J. MARTIN, AND W. M. FORD. 2015. Incidental captures of eastern spotted skunk in a high-elevation red spruce forest in Virginia. Notes of the Northeastern Naturalist 22:2.

DRAGOO, J. W., K. E. COAN, K. A. MOORE, S. E. HENKE, R. C. FLEISCHER, AND S. M. WISELY. 2009. Polymorphic microsatellite markers for the striped skunk, *Mephitis mephitis*, and other mephitids. Molecular Ecology Resources 9:383-385.

DRAY, S., AND A. B. DUFOUR. 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software 22:1-20.

EARL, D. A., AND B. M. VONHOLDT. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetic Resources 4:359-361.

EASTERN SPOTTED SKUNK COOPERATIVE STUDY GROUP. 2017. Eastern spotted skunk conservation plan – draft 1.

EVANNO, G., S. REGNAUT, AND J. GOUDET. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611-2620.

FERGUSON, A. W., M. M. MCDONOUGH, G. I. GUERRA, M. RHEUDE, J. W. DRAGOO, L. K. AMMERMAN, AND R. C. DOWLER. 2017. Phylogeography of a widespread small carnivore, the western spotted skunk (*Spilogale gracilis*) reveals temporally variable signatures of isolation across western North America. Ecology and Evolution 1-12.

FLOYD, C. H., ET AL. 2011. Genetic differentiation of island spotted skunks, *Spilogale gracilis amphiala*. Journal of Mammalogy 92:148-158.

GOMPPER, M. E., AND H. M. HACKETT. 2005. The long term, range-wide decline of a once common carnivore: the eastern spotted skunk (*Spilogale putorius*). Animal Conservation 8:195-201.

GOMPPER, M. E., AND D. S. JACHOWSKI. 2016. *Spilogale putorius*, eastern spotted skunk. In: IUCN 2017. The IUCN Red List of Threatened Species. Version 2017-1. www.iucnredlist.org. Accessed 1 June 2017.

GROBLER, J. P., G. B. HARTL, N. GROBLER, A. KOTZE, K. BOTHA, AND R. TIEDEMANN. 2005. The genetic status of an isolated black wildebeest (*Connochaetes gnou*) population from the Abe Bailey Nature Reserve, South Africa: microsatellite data on a putative past hybridization with blue wildebeest (*C. taurinus*). Mammalian Biology 70:35-45.

HACKETT, H. M., D.B. LESMEISTER, J. DESANTY-COMBES, W. G. MONTAGUE, J. J. MONTAGUE, J. J. MILLSPAUGH, AND M. E. GOMPPER. 2007. Detection rates of eastern spotted skunks (*Spilogale putorius*) in Missouri and Arkansas using live-capture and non-invasive techniques. American Midland Naturalist 158:123-131.

HAAG, T., ET AL. 2010. The effect of habitat fragmentation on the genetic structure of a top predator: loss of diversity and high differentiation among remnant populations of Atlantic Forest jaguars (*Panthera onca*). Molecular Ecology 19:4906-4921.

HARDY, L. M. 2013. The eastern spotted skunk (*Spilogale putorius*) at the Ouchita Mountains Biological Station, Polk County, Arkansas. Journal of the Arkansas Academy of Science 67:59-65.

HAYES, J. P., AND R. G. HARRISON. 1992. Variation in mitochondrial DNA and the biogeographic history of woodrats (*Neotoma*) of the eastern United States. Systematic Biology 41:331-344.

HENRY, R., D. MIQUELLE, T. SUGIMOTO, D. R. MCCULLOUGH, A. CACCONE, AND M. A. RUSSELLO. 2009. *In situ* population structure and *ex situ* representation of the endangered Amur tiger. Molecular Ecology 18:3173-3184.

IUDICA, C. A., W. M. WHITTEN, AND N. H. WILLIAMS. 2001. Small bones from dried mammal museum specimens as a reliable source of DNA. BioTechniques 30:732-736.

JAKOBSSON, M., AND N. A. ROSENBERG. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801-1806.

JONES, K. L., D. H. VAN VUREN, M. B. MCEACHERN, K. R. CROOKS, J. W. DRAGOO, AND B. MAY. 2013. Spatial and genetic organization of the island spotted skunk, *Spilogale gracilis amphiala*. Southwestern Naturalist 58:481-486.

KAPLAN, J. B., AND R. A. MEAD. 1991. Conservation status of the eastern spotted skunk. Mustelid and Viverrid Conservation 4:15.

KINLAW, A. 1995. *Spilogale putorius*. Mammalian Species 511:1-7.

KINLAW, A., L. M. EHRHART, P. D. DOERR, K. P. POLLOCK, AND J. E. HINES. 1995. Population estimate of spotted skunks (*Spilogale putorius*) on a Florida barrier island. Florida Scientist 58:47-54.

KYLE, C. J., R. D. WEIR, N. J. NEWHOUSE, H. DAVIS, AND C. STROBECK. 2004. Genetic structure of sensitive and endangered northwestern badger populations (*Taxidea taxus taxus* and *T. t. jeffersonii*). Journal of Mammalogy 85:633-639.

LESMEISTER, D. B., M. E. GOMPPER, AND J. J. MILLSPAUGH. 2008. Summer resting and den site selection by eastern spotted skunks (*Spilogale putorius*) in Arkansas. Journal of Mammalogy 89:1512-1520.

LESMEISTER, D. B., M. E. GOMPPER, AND J. J. MILLSPAUGH. 2009. Habitat selection and home range dynamics of eastern spotted skunks in the Ouachita Mountains, Arkansas, USA. Journal of Wildlife Management 73:18-25.

LESMEISTER, D. L., R. S. CROWHURST, J. J. MILLSPAUGH, AND M. E. GOMPPER. 2013. Landscape ecology of eastern spotted skunks in habitats restored for red-cockaded woodpeckers. Restoration Ecology 21:267-275.

MCCULLOUGH, C. R., AND E. K. FRITZELL. 1984. Ecological observations of eastern spotted skunks on the Ozark Plateau. Transactions of the Missouri Academy of Science 18:25-32.

MCMANUS, J. S., D. L. DALTON, A. KOTZÉ, B. SMUTS, A. DICKMAN, J. P. MARSHAL, AND M. KEITH. 2015. Gene flow and population structure of a solitary top carnivore in a human-dominated landscape. Ecology and Evolution 5:335-344.

MORITZ, C. 1994. Defining 'evolutionarily significant units' for conservation. Trends in Ecology and Evolution 9:373-375.

MUNGUIA-VEGA, A., M. RHEUDE, AND M. CULVER. 2009. Ten new microsatellite loci for the striped skunk (*Mephitis mephitis*). Conservation Genetic Resources 1:437-439.

NEAR T. J., L. M. PAGE, AND R. L. MAYDEN. 2001. Intraspecific phylogeography of *Percina evides* (Percidae: Etheostomatinae): an additional test of the Central Highlands pre-Pleistocene vicariance hypothesis. Molecular Ecology 10:2235–2240.

OKSANEN, J., ET AL. 2017. Vegan: community ecology package. R package version 2.4-3. https://CRAN.R-project.org/package=vegan.

PEAKALL, R., AND P. E. SMOUSE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

PEAKALL, R., AND P. E. SMOUSE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics 28:2537-2539.

PRITCHARD, J. K., M. STEPHENS, AND P. DONNELLY. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

R CORE TEAM. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

RAMASAMY, R. K., S. RAMASAMY, B. B. BINDROO, AND V. G. NAIK. 2014. STRUCTURE PLOT: a program for drawing elegant STRUCTURE bar plots in user friendly interface. SpringerPlus 3:431.

RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

REED, A. W., AND M. L. KENNEDY. 2000. Conservation status of the eastern spotted skunk *Spilogale putorius* in the Appalachian Mountains of Tennessee. American Midland Naturalist 144:133-138.

RILEY, S. P. D., J. P. POLLINGER, R. M. SAUVAJOT, E. C. YORK, C. BROMLEY, T. K. FULLER, AND R. K. WAYNE. 2006. A southern California freeway is a physical and social barrier to gene flow in carnivores. Molecular Ecology 15:1733-1741.

SCHWALM, D., L. P. WAITS, AND W. B. BALLARD. 2014. Little fox on the prairie: genetic structure and diversity throughout the distribution of a grassland carnivore in the United States. Conservation Genetics 15:1503-1514.

SCHWARTZ, M. K., K. RALLS, D. F. WILLIAMS, B. L. CYPHER, K. L. PILGRIM, AND R. C. FLEISCHER. 2005. Gene flow among San Joaquin kit fox populations in a severely changed ecosystem. Conservation Genetics 6:25-37.

SIKES, R. S., AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2016. 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663-688.

SMITH, O., AND J. WANG. 2014. When can noninvasive samples provide sufficient information in conservation genetics studies? Molecular Ecology Resources 14:1011-1023.

SMOUSE, P. E., AND R. PEAKALL. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity 82:561-573.

SOLTIS, D. E., A. B. MORRIS, J. S. MCLACHLAN, P. S. MANOS, AND P. S. SOLTIS. 2006. Comparative phylogeography of unglaciated eastern North America. Molecular Ecology 15:4261-4293.

SUENAGA, E., AND H. NAKAMURA. 2005. Evaluation of three methods for effective extraction of DNA from human hair. Journal of Chromatography B 820:137-141.

TALBOT, B., D. GARANT, S. ROUIX PAQUETTE, J. MAINGUY, AND F. PELLETIER. 2012. Lack of genetic structure and female-specific effect of dispersal barriers in a rabies vector, the striped skunk (*Mephitis mephitis*). PLoS ONE 7: e49736. doi: 10.1371/journal.pone.0049736.

TRUJILLO, A. L., AND E. A. HOFFMAN. 2016. Uncovering discordance between taxonomy and evolutionary history in Florida raccoons. Systematics and Biodiversity. doi: 10.1080/14772000.2016.1214190.

U.S. FISH AND WILDLIFE SERVICE. 2012. Endangered and threatened wildlife and plants; 90-day finding on a petition to list the prairie gray fox, the plains spotted skunk, and a distinct population segment of the Mearn's eastern cottontail in east-central Illinois and western Indiana as endangered or threatened species. Federal Register 77:71159-71771.

VAN GELDER, R. G. 1959. A taxonomic revision of the spotted skunks (genus *Spilogale*). Bulletin of the American Museum of Natural History 117:229-392.

VAN OOSTERHOUT, C., W. F. HUTCHINSON, D. P. M. WILLIS, AND P. SHIPLEY. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535-538.

WEBB, S. D. 1974. Pleistocene mammals of Florida. University Presses of Florida. Gainesville, Florida.

WILLOUGHBY, J. R., ET AL. 2015. The reduction of genetic diversity in threatened vertebrates and new recommendations regarding IUCN conservation rankings. Biological Conservation 191:495-503.

WRIGHT, S. 1984. Evolution and the genetics of populations. Experimental results and evolutionary deductions. University of Chicago Press. Chicago, Illinois.

Appendix 1.—Genotypes for all eastern spotted skunks (*n* = 81) across 11 microsatellite loci. U = unknown.

Tissue no.	Mel1	Meme5	Meme20	Meme75	Meph22-14	Meph22-16	Meph22-26	Meph22-70	Meph42-25	Meph42-73	nRIO-08
S. p. interrupta											
ACUNHC1957	272/272	190/194	135/135	146/154	232/232	320/320	230/238	207/207	201/207	160/160	141/157
ASK4529	258/266	194/194	120/135	146/160	232/232	320/320	224/232	205/211	207/209	160/160	141/141
ASK4856	268/276	190/192	120/120	154/154	232/232	320/320	226/226	209/213	209/211	160/166	141/141
ASK4858	266/274	192/198	120/135	146/156	232/232	320/320	230/232	197/209	207/215	160/160	141/141
ASK6142	258/266	192/198	135/135	146/158	232/232	320/320	224/224	211/217	207/209	160/164	141/143
ASK6824	272/276	192/192	135/135	146/162	232/232	320/320	224/236	207/207	207/207	160/160	141/143
ASK7225	266/270	192/192	120/120	158/160	232/232	320/320	226/234	207/215	207/207	160/160	141/141
ASK7809	266/276	192/196	135/135	152/162	232/232	320/320	224/236	207/207	207/207	162/166	141/157
ASK7814	266/266	192/196	135/135	152/154	232/232	320/320	224/232	205/209	201/207	160/168	141/141
ASK7874	268/274	192/196	135/135	150/156	232/232	320/320	230/232	193/193	209/209	160/166	141/141
ASK7931	264/274	194/196	135/135	158/162	232/232	318/318	U	193/193	209/209	160/162	141/141
ASK9618	268/270	192/194	120/120	146/162	232/232	320/320	224/228	197/207	203/207	162/162	141/143
ASK9654	264/270	198/198	120/120	156/160	232/232	320/320	224/234	193/201	207/207	160/168	141/141
ASK9686	264/264	192/192	135/135	146/146	232/232	320/320	226/234	193/205	209/215	160/166	141/141
ASK10925	268/272	192/192	120/120	146/158	232/232	320/320	224/232	209/209	207/207	160/160	141/141
ASK10926	268/272	190/192	135/135	146/152	232/232	320/320	232/236	205/205	207/207	160/160	141/141
ASK11870	266/268	192/194	120/120	160/162	232/232	320/320	228/228	187/203	207/207	164/168	143/159
ASK11871	268/268	190/192	120/120	158/158	232/232	320/320	228/234	187/211	207/207	160/164	141/143
ASK11872	266/266	190/192	135/135	156/160	232/232	320/320	228/236	187/207	207/218	152/166	141/141
ASK11873	266/266	190/192	135/135	160/160	232/232	318/320	230/238	199/199	U	160/160	141/141
ASK11881	266/266	192/194	120/135	158/160	232/232	318/320	228/236	187/193	207/218	166/168	141/141
ASK11884	264/272	192/194	135/135	146/148	232/232	320/320	232/232	205/207	207/209	160/168	141/155
ASK11912	266/274	192/U	120/120	156/156	232/232	320/320	230/230	193/205	207/209	160/164	141/143
ASK11913	266/268	192/194	135/135	146/162	232/232	320/320	234/234	197/207	201/207	160/168	141/143
ASK11914	268/270	192/192	135/135	158/164	232/232	320/320	230/234	193/207	207/207	162/168	141/159
ASK11915	266/266	192/196	135/135	160/164	232/232	320/320	226/230	199/215	207/218	168/168	141/141

Appendix 1.—Continued

Tissue no.	Mel1	Meme5	Meme20	Meme75	Meph22-14	Meph22-16	Meph22-26	Meph22-70	Meph42-25	Meph42-73	nRIO-08
ASK11916	272/276	192/194	135/135	162/162	230/232	320/320	236/240	207/215	201/207	160/166	157/157
ASK12461	266/268	194/194	120/120	158/160	232/232	320/320	228/236	203/203	201/209	160/164	141/141
ASK12462	266/268	194/194	120/120	158/162	232/232	320/320	228/228	185/195	201/207	160/160	141/141
ASK12480	274/274	194/194	135/135	146/154	232/232	320/320	224/232	193/215	201/207	160/168	141/141
ASK12482	274/274	192/198	135/135	146/162	232/232	320/320	232/232	209/211	201/207	160/160	141/141
ASK12490	268/274	192/196	120/120	146/162	232/232	320/320	232/240	201/203	201/207	160/166	141/157
ASK12491	258/268	192/194	135/135	146/156	232/232	320/320	236/236	213/215	207/207	158/158	141/141
ASK12693	266/272	190/192	135/135	146/158	232/232	320/320	226/238	205/213	201/207	160/160	141/143
TCWC60748	266/276	192/192	120/120	146/152	232/232	320/320	224/230	193/205	209/215	160/160	141/141
TK29908	266/276	192/194	120/135	158/158	232/232	320/320	232/238	207/209	207/215	160/160	143/143
S. p. putorius											
ACC1139	272/272	176/178	135/135	150/150	246/246	320/320	220/238	205/209	220/220	158/164	147/147
ASK11910	270/272	176/188	135/135	150/152	246/248	320/320	236/236	193/201	218/220	158/160	147/147
ASK11911	270/272	176/176	135/135	150/150	246/246	320/322	232/236	193/209	220/220	160/160	147/151
ASK12466	272/272	176/176	135/135	150/178	232/246	320/320	220/238	193/211	220/222	158/160	147/147
ASK12467	272/272	176/188	135/135	150/152	246/246	320/320	220/230	191/195	220/220	160/162	147/147
ASK12468	272/274	176/176	135/135	150/152	246/246	320/320	220/220	205/205	222/224	158/162	145/147
JJK3648	268/270	176/178	135/135	152/152	232/246	320/320	220/U	197/199	218/222	158/160	147/147
JJK3857	272/272	176/176	135/135	150/150	246/246	320/320	236/236	191/199	222/222	160/162	145/147
UWG215	270/272	178/178	135/135	150/150	246/250	322/324	220/220	209/209	218/222	158/160	147/147
UWG305	270/274	176/178	135/135	150/178	246/246	320/324	232/240	195/211	215/222	158/160	145/147
UWG308	272/272	176/178	135/135	150/150	246/246	320/320	222/234	193/211	222/222	158/164	147/147
UWG355	270/274	176/176	135/135	150/178	246/246	322/322	220/234	191/191	218/220	158/158	147/147
UWG389	270/274	176/176	135/135	178/178	232/246	320/320	236/236	191/201	218/218	162/162	147/147
UWG424	274/274	176/176	135/135	150/178	232/246	320/320	232/236	191/211	218/222	160/162	147/147
UWG525	272/272	176/176	135/135	150/152	246/246	320/320	232/236	191/199	220/222	158/160	147/147
UWG585	270/274	176/176	135/135	150/178	246/246	322/322	220/232	191/191	218/220	158/158	147/147

Appendix 1.—Continued

Tissue no.	Mel1	Meme5	Meme20	Meme75	Meph22-14	Meph22-16	Meph22-26	Meph22-70	Meph42-25	Meph42-73	nRIO-08
UWG615	270/272	176/176	135/135	150/150	246/246	320/320	220/236	199/207	222/222	158/158	147/147
UWG645	272/276	176/176	135/135	150/150	246/246	320/320	220/238	199/207	218/222	158/166	147/147
UWG695	272/274	176/176	135/135	150/150	246/248	320/322	220/220	191/211	218/220	158/158	147/147
UWG865	272/272	176/176	135/135	150/150	246/250	320/324	220/232	191/191	220/222	158/158	147/147
WFB8979	270/270	176/176	135/135	150/150	246/246	320/320	232/236	191/209	201/220	158/164	147/149
S. p. ambarvalis											
FWC02	270/274	176/192	135/135	150/150	256/256	318/318	232/236	193/203	226/236	162/164	139/141
FWC06	268/270	178/178	135/135	150/150	246/246	318/318	228/232	195/195	218/236	160/166	139/147
FWC12	274/274	176/194	135/135	150/150	246/246	318/318	228/232	191/195	215/236	164/166	141/141
FWC14	272/274	178/194	135/135	150/152	244/256	318/318	228/236	207/207	226/226	160/160	141/143
FWC15	270/270	176/176	135/135	150/152	230/232	318/318	U	195/211	220/226	160/166	141/143
FWC16	268/274	176/194	135/135	150/150	230/246	318/318	232/232	195/195	222/236	164/166	141/141
FWC17	270/274	176/194	135/135	150/150	246/246	328/328	230/232	195/209	215/228	160/164	141/141
FWC18	268/270	176/178	135/135	150/150	244/246	320/328	232/234	195/207	226/228	166/168	143/143
FWC19	268/270	178/194	135/135	150/150	246/246	322/328	228/230	195/195	218/236	164/164	139/143
FWC20	268/274	178/178	135/135	150/150	246/246	318/328	228/232	195/195	203/222	164/168	141/143
FWC22	270/274	188/192	135/135	150/152	246/256	318/318	222/230	207/207	226/228	160/164	139/139
FWC24	270/274	176/178	135/135	150/152	232/246	318/320	228/230	195/207	220/220	160/164	139/141
FWC26	272/276	178/192	135/135	150/150	246/256	320/320	228/228	195/205	226/226	160/162	143/143
FWC27	272/276	188/194	135/135	150/150	246/246	318/318	222/232	195/205	203/228	160/168	141/141
FWC28	270/278	176/194	135/135	150/152	244/246	320/322	222/236	195/203	203/218	160/164	139/139
FWC29	270/274	176/188	135/135	150/150	244/256	318/328	U	193/207	218/218	168/168	139/139
FWC30	274/276	178/188	135/135	150/150	232/246	322/328	228/236	193/201	218/220	162/164	141/143
FWC32	268/268	178/178	135/135	150/150	230/246	322/328	232/236	195/195	203/222	164/166	141/143
FWC40	274/276	176/188	135/135	150/150	232/246	320/322	U	193/207	U	160/160	139/141
FWC41	272/274	192/194	135/135	150/150	244/246	328/328	228/230	205/207	215/236	162/168	139/147
FWC42	270/274	176/178	135/135	150/152	232/246	318/320	228/230	195/207	220/220	160/164	139/141

Appendix 1.—Continued

	Mel1	Meme5	Meme20	Meme75	Meph22-14	Meph22-16	Meph22-26	Meph22-70	Meph42-25	Meph42-73	nRIO-08
FWC49	270/272	176/194	135/135	150/150	244/246	318/320	230/236	207/209	220/226	164/168	139/141
FWC50	270/270	176/192	135/135	150/152	230/232	318/318	228/230	195/207	218/218	162/164	139/141
FWC57	272/276	188/194	135/135	150/150	246/246	318/318	222/232	195/205	203/228	158/166	141/141